via four G-protein-coupled receptors, EP1-EP4. We investigated role of PGE2 on mitochondrial function and matrix degradation in chondrocytes.
Methods: Cartilage explants were prepared from OA patients undergoing joint replacement surgery. In selected experiments chondrocytes were isolated using collagenase digestion and cultured in alginate beads. PGE2 receptor (EP1-4) expression was analyzed by FACS. Mitochondrial function was assessed by the fluorescent probe JC-1 and ATP generation.
Results: Immunostaining for enzymes COX-1, COX-2, cPGES and mPGES confirmed the presence of PG biosynthetic pathway(s) in OA cartilage. All four PGE2 EP receptors were expressed in both normal and OA cartilage and confirmed in OA chondrocytes by FACS. However, the EP4 receptor was upregulated (2-3-fold) in OA compared to normal. Addition of PGE2 (0.1-10uM) exerted the following deleterious effects on OA chondrocytes: 1) decreased mitochondrial membrane activity (fluorescent probe JC-1) and ATP generation 2) inhibition of proteoglycan synthesis (^{35}S incorporation) by chondrocyte cultures to levels of 75% of control ($p<0.01$), 3) increased type II collagen degradation as assessed by C12C ELISA. 4) inhibited expression of aggrecan and type II collagen mRNA and 5) induced production of pro-MMP-13, IL-6 and IL-8, but inhibited MMP-1 secretion. The potency of PGE2 effects were comparable to that of IL-1b, and the inhibitory effects of a combination of IL-1 and PGE2 (10uM) were additive.To examine the effects of endogenous PGE2 produced in response to IL-1, we performed experiments in the presence or absence of the EP4 antagonist, A23858 or COX-2 selective inhibitor celecoxib. The addition of IL-1 reduced chondrocyte proteoglycan synthesis to 45% of control values; pretreatment with 10uM A23858 or 2uM celecoxib, reversed this inhibition to 67% and 65% respectively of control values ($p<0.01$). Both A23858 and celecoxib reversed other catabolic effects of IL-1 (1-4 above), including mitochondrial activity, ATP generation and inhibition of PGE2 dependent production of MMP-13.
Conclusions: PGE2, the predominant eicosanoid produced by OA chondrocytes, exerts catabolic effects within cartilage. These deleterious effects are mediated, in part via the EP4 receptor, which should be considered as a potential target for disease modifying agents in OA.

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EFFECT OF 4-HYDROXYNONENAL ON INFLAMMATORY RESPONSES IN HUMAN OSTEOARTHRITIC CARTILAGE

M. Benderdour, F. Vaillancourt, Q. Shi, P. Lavigne, J. Fernandes
University of Montreal, Montreal, PQ, Canada

Purpose: We have recently reported the catabolic role of 4-hydroxynonenal (HNE), a lipid peroxidation end-product, in osteoarthritic (OA) cartilage. In this study, we provide additional evidence that HNE acts as an inflammatory mediator by elucidating the signaling cascades targeted in OA chondrocytes.
Methods: Levels of prostaglandin E2 (PGE_2) and nitric oxide (NO) levels were measured respectively by specific enzyme immunosorbent assay and by the Griess reaction. Expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) was evaluated by western blotting and semi-quantitative reveres transcriptase polymerase chain reaction. COX-2 and iNOS promoter activity was analyzed in transient transfection experiments. The formation of the HNE/IKKα adducts was measured by immunoprecipitation.
Results: HNE induced COX-2 protein and mRNA levels with accompanying increases in PGE_2 production. In contrast, HNE had no effect on iNOS expression or NO release. However, HNE strongly inhibited IL-1β-induced iNOS expression or NO production. Transient transfection experiments revealed that the ATF/CRE site (-58/-53) is essential for HNE-induced COX-2 promoter activation and indeed HNE induced ATF-2 and CREB-1 phosphorylation as well as ATF/CRE binding activity. Overexpression of p38 MAPK enhanced the HNE-induced ATF/CRE activation, COX-2 synthesis and promoter activity. HNE abrogated IL-1β-induced iNOS expression and promoter activity mainly through NF-κB site (-5 817/-5 808) via suppression of IκBα phosphorylation and NF-κB/p65 nuclear translocation. Upon examination of upstream signaling components, we found that IKKα was inactivated through HNE/IKKα adduct formation.
Conclusions: Taken together, these findings illustrate the central role played by HNE in the regulation of COX-2 and iNOS in OA. The aldehyde induced selectively COX-2 via ATF/CRE activation and prevented iNOS expression via IKKα inactivation

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ACUTE INTRA-ARTICULAR HAEMARTHROSIS: A REAL JEOPARDY

B. Fodor
Department of Traumatology and Hand Surgery, Faculty of Medicine, University of Pécs,Hungary, Pécs, Hungary

Purpose: Flow cytometric analysis of leukocytes derived from intra-articular effusions of patients with acute knee injury revealed enhanced expressions of inflammation associated cell surface antigens.
These results draw the attention to the general cytokine overload in consequence of a notable leukocyte activation, which affects the whole intra-articular environment. Thus our study aims to assess the cytokine compound of intra-articular effusion derived from acute haemarthrotic patients in order to estimate the their effects on the joint.
Methods: Paired samples of peripheral blood and knee joint effusions were obtained from 40 patients, all males and ranging from 15 to 45 (mean 30) years in age, following 15$\pm$10 hours after the sport injury. Flow cytometric measurements of IL-1, IL-6, IL-8, IL-10, IL-12 and TNF-alpha levels were performed on the samples and the results were compared statistically.
Results: Significantly increased levels of IL-1 (effusion 1770,10$\pm$320,70 pg/ml; venous blood: 140,65$\pm$35,60 pg/ml;), IL-6 (effusion 8180,45$\pm$677,65 pg/ml; venous blood: 370,42$\pm$81,84 pg/ml;), IL-8 (effusion 2081,24$\pm$443,1 pg/ml; venous blood: 90,2$\pm$22,3 pg/ml;), IL-12 (effusion 35,22$\pm$6,74 pg/ml; venous blood: 24,6$\pm$6,5 pg/ml) and TNF-alpha (effusion 13,22$\pm$2,83 pg/ml; venous blood: 7,88$\pm$1,5 pg/ml) in samples derived from injured knees, whereas IL-10 (effusion 51,12$\pm$11,19 pg/ml; venous blood: 43,24$\pm$11,12) showed only discrete, non significant elevation.
Conclusions: The highly elevated cytokine levels measured during our recent clinical investigations provide further evidence for a robust inflammation in case of acute haemarthrosis. Since the concentrations of such agents are comparable to the cytokine levels measured in different clinical cases of inflammation.

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EXPRESSION PATTERNS OF NOTCH RECEPTORS, THEIR LIGANDS AND HES5 IN HUMAN ARTICULAR CARTILAGE

C. Brantsing, C. Karlsson, A. Lindahl
Biomedicine, Molecular Cellbiology, Gothenburg, Sweden

Purpose: Notch family proteins are transmembrane receptors that control cell fate and proliferation. Osteoarthritis (OA) is characterized by activation and abnormal proliferation/differentiation of chondrocytes. We therefore examined the expression markers